strontium. Thus, all of the materials, particularly the sodium titanosilicate, have good potential for the decontamination of high-salt, alkaline nuclear wastes.

Conclusions

Inorganic ion exchangers have a wide number of applications within the nuclear industry and are preferred over conventional organic resins. Zeolites are ideal for the treatment of dilute wastes, provided that the pH is not too extreme, and their relatively low costs make their use highly economical. For more extreme wastes like those encountered in the Hanford storage tanks, new titanium-based materials have been developed that are able to withstand the high alkalinity and have sufficiently high selectivity to remove trace levels of strontium in the presence of molar quantities of other ions. Although these synthetic exchangers cost hundreds of US dollars per kilogram, their extreme selectivity and ability to be regenerated makes them viable options for the treatment of these extremely complex wastes.

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See also: **I/Ion Exchange. II/Ion Exchange:** Catalysis: Organic Ion Exchangers; Historical Development; Inorganic Ion Exchangers; Novel Layered Materials: Non-Phosphates; Novel Layered Materials: Phosphates; Organic Ion Exchangers; Surface Complexation Theory: Multispecies Ion Exchange Equilibria; Theory of Ion Exchange.

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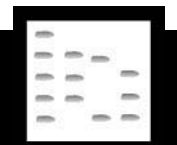
SUB-CRITICAL WATER: EXTRACTION

See **III / SUPERCRITICAL FLUID EXTRACTION-SUPERCRITICAL FLUID CHROMATOGRAPHY**

SUGAR DERIVATIVES: CHROMATOGRAPHY



S. C. Churms, University of Cape Town, South Africa

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Because sugar derivatives are generally present as complex mixtures, chromatographic techniques are crucial in their analysis. The spectrophotometric methods, and other methods mentioned in this article, serve primarily as chromatographic detection systems, and spectroscopic methods are frequently used in conjunction with chromatography.

Detection Reagents for Planar Chromatography and for Qualitative and Spot Tests

Detection reagents that are specific for particular derivatives, or can distinguish certain classes from

others, are listed in **Table 1**, which also shows the colours produced in each case and, where known, the detection limit. These reagents are used in spot tests and as detection reagents in planar chromatography, those not containing corrosive acids being applicable to both paper and thin-layer chromatography.

Gas Chromatography

Some sugar derivatives are sufficiently volatile to be analysed by gas chromatography (GC) without further derivatization; this particularly applies to the partially methylated methyl glycosides and methyl glycoside methyl esters produced by methanolysis of methylated polysaccharides. Multiple peaks, corresponding to α and β anomers of pyranoside and furanoside forms, are given by each glycoside, a factor that complicates analysis but can aid the identification of the individual components of simple, well-resolved mixtures. Unsubstituted methyl glycosides require derivatization for analysis by GC; they are successfully analysed as either trimethylsilyl (TMS) ethers or trifluoroacetyl (TFA) esters. Here again the characteristic patterns of multiple peaks produced can facilitate identification. However, for analysis of complex mixtures it is desirable to simplify the chromatogram by elimination of the anomeric centre.

The mixtures of partially methylated sugars obtained in methylation analysis of polysaccharides are usually submitted to GC as the acetylated alditol derivatives, for which a large body of mass spectrometry (MS) data is available. However, for some carbohydrates, notably amino- and acetamidodeoxy sugars, the GC retention times of the derived alditol acetates are excessively long. For aminodeoxyhexoses this problem can be overcome by nitrous acid deamination of the amino sugars before reduction and acetylation, or by *N*-methylation of the aminodeoxyalditols prior to acetylation. The most satisfactory procedure in the analysis of the mixtures of sugars obtained on hydrolysis of bacterial cell wall polysaccharides or glycoconjugates is derivatization to *O*-methyloximes, followed by acetylation or trimethylsilylation. No more than two peaks are produced by each component of the mixture and simultaneous analysis of neutral and amino sugars, as well as *N*-acetylneuraminic acid, muramic acid and its *N*-acetyl derivative and 3-deoxy-D-manno-2-octulosonic acid (KDO), within 40 min is possible by capillary GC as the acetylated *O*-methyloximes.

GC analysis of uronic acids also requires derivatization by specific methods if the multiple peaks given by methyl glycoside methyl esters or TMS ethers are to be avoided. Conversion to the oxime is an option in this case too, or the acids may be reduced to aldonic acids (by sodium borohydride reduction of the alduronates) and analysed as the TMS derivatives of the aldonolactones or the acetylated derivatives of the *N*-alkylaldonamides produced on reaction of the aldonolactones with a L-alkylamine in pyridine. Both methods of derivatization proceeding via the aldonic acids result in the production of a single GC peak for each uronic acid present. The latter method has the advantage that simultaneous analysis of aldoses, as the alditol acetates, is possible – the alditol acetates have much shorter retention times than the *N*-alkylaldonamide acetates. Complete analysis of neutral and acidic sugars within 20 min is possible by capillary GC of these derivatives.

Oligosaccharide-alditols, up to tetrasaccharide level, can be analysed by GC as their permethylated derivatives. The volatility of those containing acetamidodeoxyhexose residues can be increased by *N*-trifluoroacetylation of these residues (through transamidation by trifluoroacetolysis under carefully controlled conditions) prior to methylation. This procedure permits GC analysis of oligosaccharide-alditols containing up to seven sugar residues and also allows the use of the electron capture detector, with a hundredfold increase in sensitivity.

Recommended conditions for GC analysis of various sugar derivatives are listed in **Table 2**. Comprehensive retention data are available in the literature.

Liquid Chromatography

Carbohydrate derivatives can be analysed by liquid chromatography (LC) in various modes, depending on the polarity of the molecule and whether acidic or basic groups are present. Nonpolar compounds, or those rendered nonpolar by derivatization to increase the sensitivity of analysis, are amenable to reversed-phase LC or adsorption chromatography on silica. For hydroxylic compounds such as alditols, several options are available, including normal-phase LC on bonded amino phases or amine-modified silica (the column packing being modified *in situ* by addition of a polyfunctional amine to the mobile phase); LC on a cation exchange resin in the Ca^{2+} form (ion-moderated partitioning) or, as borate complexes, on an anion exchange resin; and ion chromatography, with pulsed amperometric detection. Recently, cyclodextrin-bonded silica has also proved effective.

The oligosaccharide-alditols obtained in degradative structural studies of glycoproteins can also be analysed by LC in various ways; normal-phase LC, ion exchange, ion chromatography and size exclusion chromatography. Amino- and acetamidodeoxyhexoses and the hexitols derived from them can be analysed by normal-phase LC; ion-moderated

Table 1 Reagents used in qualitative analysis for detection of sugar derivatives

Derivatives	*Reagent mixture*	*Procedure*	*Colour produced*
Alditols and cyclitols	Fleury's reagent: (a) HgO (5%, m/v) in HNO_3 (5%, m/v), diluted 1 : 1 before use (b) Barium acetate (10%, m/v) mixed 1 : 10 with glacial acetic acid	Spray with (a); heat at 90–100°C for 10 min; spray with (b); heat at 100°C for 10–30 min	Specific for polyols; cyclitols give orange spots (detection limit 5–10 μg inositol); alditols and other polyols grey to black
	Periodate-*p*-anisidine: (a) *p*-Anisidine (1%, m/v) in ethanol (70%, v/v) (b) $NaIO_4$ (0.1 mol L^{-1} aqueous solution) mixed 1 : 10 with acetone	Spray with (a); heat at 105°C for 5–10 min; dip in (b)	Polyols and aldonic acids give white spots on brown background; distinguished from aminodeoxy sugars, uronic acids and neutral sugars (see below)
	Vanillin–perchloric acid: (a) Vanillin (1%, m/v) in ethanol (b) $HClO_4$ (3% aqueous solutioin). Mixed 1 : 1 with (a) before use	Spray; heat at 85°C for 5–10 min	Polyols give pale blue to lilac spots (detection limit 20–30 μg); cyclitols and most aldoses do not react, ketoses give grey-green spots, rhamnose brick-red
Aldonic acids and aldonolactones	Periodate-*p*-anisidine: see above	See above	Aldonic acids give white spots on brown background (see above)
	Hydroxylamine–iron (III) chloride (a) Hydroxylamine hydrochloride (1 mol L^{-1}) in methanol (b) KOH (1.1 mol L^{-1}) in methanol (c) $FeCl_3$ (2%, m/v) in HCl (1% aqueous solution)	Spray with 1 : 1 mixture of (a) and (b); dry at room temperature for 10 min; spray with (c)	Aldonolactones revealed as purple spots; other lactones and esters also react
Amino- and acetamidodeoxy sugars	Elson–Morgan reagent (a) KOH (25%, m/v) in aqueous ethanol (80%, v/v) (b) Pentane-2,4-dione (acetylacetone), redistilled (1%, v/v) in 95% ethanol, fresh solution (c) *N*,*N*-Dimethyl-*p*-aminobenzaldehyde (10%, m/v) in conc. HCl (d) Ethanol (95%)	Dip through mixture of (a) and (b) (1 : 10); heat at 110°C for 5 min; dip through mixture of (c) and (d) (1 : 1); dry in stream of cold air	Specific for amino- and acetamidodeoxyhexoses. Transient purple spots at room temperature; heating to 80°C gives permanent red spots for free aminodeoxy sugars, purple-violet for acetamidodeoxy sugars
	Fluorescamine (a) Triethylamine (10%, v/v) in dichloromethane (b) Fluorescamine (0.05%, m/v) in acetone	Spray with (a); dry in air; spray with (b); dry in air; spray again with (a)	Specific for amino- and acetamidodeoxy sugars. Fluorimetric scanning (excitation 390 nm, emission 475 nm) detects amino sugars (detection limit 100 pmol)
	Ninhydrin 0.1% (m/v) solution in 1-butanol	Spray, heat at 105–110°C for 10 min	Specific reagent; purple spots produced
	Periodate-*p*-anisidine See above	See above	Yellow spots on brown background
Esters and lactones	Hydroxylamine-iron (III) chloride See above	See above	Purple spots
Ketals	2,4-Dinitrophenylhydrazine 0.4% (m/v) solution in 2 mol L^{-1} HCl	Spray; heat at 105°C for 5 min	Specific for keto group; ketoses and ketals (e.g. pyruvate) give orange spots on light yellow background

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Table 1 *Continued*

Derivatives	*Reagent mixture*	*Procedure*	*Colour produced*
Methyl ethers and methyl esters	*p*-Anisidine hydrochloride 3% (m/v) solution in 1-butanol	Spray; heat at 110°C for 10 min	Methyl ethers give highly characteristic pink, red or brown spots, some fluorescent under UV. Methyl esters of methylated uronic acids give bright pink colour
Neuraminic acids	Resorcinol–HCl–Cu(II) (a) Resorcinol (0.2%, m/v) in 4 mol L^{-1} HCl (b) Aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.1 mol L^{-1}). Mix (a) and (b) (40 : 1) at least 4 h before use	Spray; heat at 110–120°C for about 20 min	Neuraminic acids give blue spots; ketoses also react
	Periodate–thiobarbiturate (a) $NaIO_4$ (0.5 mol L^{-1}) in 0.025 mol L^{-1} H_2SO_4 (b) Ethylene glycol–aetone–conc. H_2SO_4 (50 : 50 : 0.3 v/v/v) (c) Sodium 2-thiobarbiturate, 6% (m/v) in H_2O	Spray with (a); leave at room temperature for 15 min; dry thoroughly in stream of air; spray with (b); dry similarly. If odour of formaldehyde persists spray again with (b). Finally, spray with (c); heat at 100°C for 10 min	Only neuraminic acids give red spots (detection limit about 3 μg)
Phosphates	Ammonium molybdate 10% (m/v) aqueous solution (20 mL) added to conc. HCl (3 mL) with shaking; NH_4Cl (5 g) added	Spray	Phosphorylated derivatives give immediate yellow colour of ammonium phosphomolybdate
Uronic acids	*p*-Anisidine hydrochloride See above	See above	Red spots; do not fluoresce under UV
	Periodate-*p*-anisidine See above	See above	Red spots on brown background; do not fluoresce (distinction from spots given by neutral sugars)
	Mixed indicators Thymol blue (0.025%, m/v) and bromothymol blue (0.06%, m/v) in 95% ethanol; 1 mol L^{-1} NaOH added until blue-green colour reached	Spray	Uronic acids and oligomers (e.g. oligogalacturonic acids) give red spots on green background

Table 2 Conditions recommended for GC analysis of sugar derivatives

Compounds	*Derivatives for GC*	*Column type*	*Phase*	*Temperature (°C)*	*Gas; flow rate (mL min^{-1})*
Acetals, isopropylidene	Run as such	Packed	(1) OV-225, 3% on Supelcoport (100–120 mesh) (2) ECNSS-M, 3% on Gas-Chrom Q (100–120 mesh); 1 and 2 mixed 7 : 4	90 → 190°C at 4°C min^{-1}	N_2; 20
Alditols	Acetates	Packed	OV-225, 3% on Chromosorb W-HP (80–100 mesh)	210	He; 40
		Capillary	OV-225	100 → 250°C at 4°C min^{-1}	He; 1
Aminodeoxyhexoses	Alditol acetates, deaminated	Capillary	Silar 10C	190°C (4 min); 190 → 230°C at 4°C min^{-1}; 230°C (8 min)	H_2; 9
	Trifluoroacetates	Packed	OV-101, 5% on Chromosorb W AW DMCS (60–80 mesh)	120	N_2; 50
		Capillary	OV-225	70°C (2 min); 70 → 180°C at 5°C min^{-1}; 180°C (15 min)	N_2; 1.5
Aldonic and aldaric acids	TMS ethers	Packed	OV-1 or OV-17, 0.5% on Chromosorb G (100–120 mesh)	160	N_2; 30
		Capillary	OV-101	100°C (2 min); 100 → 200°C at 20° min^{-1}; 200°C (5 min)	H_2; 2
Aldonolactones	TMS ethers	Packed	OV-1 or OV-17, 0.5% on Chromosorb G (100–120 mesh)	160	N_2; 30
	Acetylated *N*-alkylaldonamides (1-propyl or 1-hexyl usual alkyl substitutents)	Packed	SP-2340, 3% on Supelcoport (100–120 mesh)	190 → 260°C at 5°C min^{-1}	He; 20
		Capillary	SP-2330	200°C (2 min); 200 → 235°C at 3°C min^{-1}; 235°C (5 min)	He; 10
		Capillary (fused silica)	DB-1701 (bonded phase)	220 → 270°C at 1°C min^{-1}	He; 12
Aminodeoxyhexoses	Alditol acetates, *N*-methylated	Packed	EGSS-X, 2% on Chromosorb W AW DMCS (60–80 mesh)	195	N_2; 45
	Alditol acetates, deaminated	Packed	SP-2340, 3% on Supelcoport (100–120 mesh)	150 → 220°C at 2°C min^{-1}	N_2; 40
		Capillary	Silar 10C	190°C (4 min); 190 → 230°C at 4°C min^{-1}; 230°C (8 min)	H_2; 9
	Alditols, trifluoroacetylated	Packed	OV-101, 5% on Chromosorb WAW DMCS (60–80 mesh)	120	N_2; 50

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Table 2 *Continued*

Compounds	*Derivatives for GC*	*Column type*	*Phase*	*Temperature (°C)*	*Gas; flow rate (mL min^{-1})*
Amino- and acetamidodeoxy-hexoses	Methyl glycosides, trifluoroacetylated	Capillary	OV-210	120 → 210°C at 1°C min^{-1}	Ar; 1
			SE-30	90°C (4 min); 90 → 250°C at 8°C min^{-1}	He; 1.5
	Methyl glycosides, trimethylsilylated	Packed	SE-30, 3% on Chromosorb W-HP (100–120 mesh)	80 → 250°C at 2°C min^{-1}	N_2; 25
		Capillary (fused silica)	CP-Sil 5	140°C (2 min); 140 → 260°C at 8°C min^{-1}	He; 1
	O-Methyloximes, acetylated	Capillary	OV-1	175°C (4 min); 175 → 260°C at 4°C min^{-1}; 260°C (5 min)	He; 0.5
	O-Methyloximes, trimethylsilylated	Capillary (fused silica)	SP-2100	180	He; 1
Anhydroalditols	TMS ethers	Capillary (fused silica)	CP-Sil 5	130 → 220°C at 2°C min^{-1}	N_2; 1.5
Cyclitols	Trifluoroacetates	Packed	Dexsil 410, 3% on Chromosorb W-HP (80–100 mesh)	100°C (1.5 min); 100 → 310°C at 3.5°C min^{-1} (3.5 min), 6°C min^{-1} (5 min), 15°C min^{-1} (5 min); 25°C min^{-1} (4 min); 310°C (6 min)	N_2; 20
	TMS ethers	Packed	SE-30, 3% on Gas-Chrom Q (80–100 mesh)	130 → 190°C at 2°C min^{-1}	N_2; 25
Methyl ethers	Alditol acetates	Packed	OV-225, 3% on Chromosorb W-HP (80–100 mesh)	175	He; 40
		Capillary (fused silica)	DB–225 (bonded phase)	195	He; 1
		Capillary	OV-275	165 → 215°C at 2°C min^{-1}; 215°C (15 min)	He; 1.5
		Capillary (fused silica)	OV-275 (bonded phase)	150 → 250°C at 4°C min^{-1}; 250°C (10 min)	He; 0.8
Methyl glycosides	Trifluoroacetates	Capillary	SE-30	90°C (4 min); 90 → 250°C at 8°C min^{-1}	He; 1.5
	TMS ethers	Packed	SE-30, 3% on Chromosorb W-HP (100–120 mesh)	80 → 250°C at 2°C min^{-1}	N_2; 25
		Capillary (fused silica)	CP-Sil 5	140°C (2 min). 140 → 160°C at 8°C min^{-1}	He; 1
		Capillary (fused silica)	DB-5 (bonded phase)	150 → 220°C at 2°C min^{-1}	N_2; 1

Table 2 *Continued*

Compounds	*Derivatives for GC*	*Column type*	*Phase*	*Temperature (°C)*	*Gas; flow rate (mL min^{-1})*
Methyl glycosides, methylated	Run as such	Packed	Ethylene glycol succinate, polyester, 14% on Chromosorb W (80–100 mesh)	155	He; 40
Muramic acid, KDO and neuraminic acid derivatives	*O*-Methyloximes, acetylated	Capillary	OV-1	175°C (4 min); 175 → 260°C at 4° min^{-1}; 260°C (5 min)	He; 0.5
Oligosaccharide-alditols	Permethylated	Packed	Dexsil 300, 1% on Supelcoport (100–120 mesh)	150 → 320°C at 4°C min^{-1}	He; 40
	Permethylated, *N*-trifluoracetylated	Capillary	OV-101	200°C (2 min); 200 → 350°C at 4°C min^{-1}	He; 0.8
Uronic acids	Methyl glycoside methyl esters, trimethylsilylated	Capillary (fused silica)	CP-Sil 5	140°C (2 min); 140 → 260°C at 8°C min^{-1}	He; 1
		Capillary (fused silica)	DB-5 (bonded phase)	150 → 220°C at 2°C min^{-1}	N_2; 1
	O-Methyloximes, trimethylsilylated	Capillary (fused silica)	SP-2100	180	He; 1
	Aldonolactones, trimethylsilylated	Packed	OV-1 or OV-17, 0.5% on Chromosorb G (100–120 mesh)	160	N_2; 30
	N-Alkylaldonamides, acetylated	Packed	SP-2340, 3% on Supelcoport (100–120 mesh)	190 → 260°C at 5°C min^{-1}	He; 20
		Capillary	SP-2330	200°C (2 min); 200 → 235°C at 3°C min^{-1}; 235°C (5 min)	He; 10
		Capilary (fused silica)	DB-1701 (bonded phase)	220 → 270°C at 1°C min^{-1}	He; 12

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partitioning on a cation exchange resin with an aqueous–organic solvent system as eluent; cation exchange chromatography; or ion chromatography. Uronic acids, on the other hand, are best analysed by anion exchange chromatography or ion-moderated partitioning on a cation exchange resin in the H^+ form. The same applies to aldonic acids and aldonolactones. Oligogalacturonic acids are similarly analysed, but ion chromatography and ion pair chromatography (with the tetrabutylammonium cation in the mobile phase) are further options in this case. The ion pair method has been applied to both normal oligogalacturonic acids and the unsaturated products (with 4,5-unsaturated residues at their nonreducing termini) given on digestion of pectic acid with *endo*-polygalacturonic acid lyase. The unsaturated acids obtained on lyase digestion of glycosaminoglycuronans can also be analysed by this method, as well as by anion exchange chromatography and ion-moderated partitioning on a cation exchange resin with an aqueous–organic solvent system.

The various LC systems applicable to analysis of carbohydrate derivatives are listed in **Table 3**. Retention data have been published elsewhere.

Electrochemical Methods Linked to LC

The pulsed amperometric detector, in which analytes are oxidized at the surface of a gold electrode, a selected potential being applied between this and a silver/silver chloride reference electrode, with a glassy carbon counterelectrode maintaining the potential without excessive drain on the reference electrode, has proved highly successful when applied in ion chromatography of carbohydrates at high pH (≥ 12). Not only neutral sugars but also alditols, amino- and acetamidodeoxyhexoses, neuraminic acid derivatives and uronic acids can be analysed in this way. If the concentration of NaOH in the eluent is too low for optimal response of the detector, post-column addition of NaOH at higher concentration is required; an example of this is the analysis of amino- and acetamidodeoxyhexoses, which are best resolved with eluents containing 10–15 mmol L^{-1} sodium hydroxide, but are only detected satisfactorily after addition of 0.3 mol L^{-1} sodium hydroxide to the column effluent. The method is applicable to oligosaccharides, including the complex series, neutral, sialylated or phosphorylated, derived from glycoconjugates, and is now extensively used in analysis of such oligosaccharides.

It is only readily oxidizable compounds that can be analysed by oxidation at the surface of a glassy carbon electrode, and this permits the determination of L-ascorbic acid in the presence of other carbohydrates that are not electroactive with this electrode. Examples include the analysis of algal extracts for L-ascorbic acid and its C5 diastereoisomer, D-erythorbic acid, at nanogram levels, after LC on a microparticulate cation exchange resin (H^+ form), eluted with 0.1 mol L^{-1} formic acid; co-eluting reducing sugars and lactones do not interfere when the carbon electrode is used as a detector. The use of this electrochemical detector has also proved invaluable in the determination of L-ascorbic acid in beers, to which it is added as an antioxidant; in a recommended procedure the glassy carbon electrode is used as a detector in LC of the beer samples on C_{18}-silica, eluted with a citrate buffer (pH 4.4) containing *N*-methyldodecylamine (1 mmol L^{-1}) as an ion-pairing reagent. The detection limit for ascorbic acid is about 1 ng.

Conductivity detectors can be used in the analysis of charged molecules. An example is afforded by the simultaneous determination of inositol phosphates, sugar phosphates and aliphatic organic anions such as pyruvate, lactate and citrate in physiological samples (rat brain and liver) by ion chromatography with conductivity detection. A post-column micromembrane suppressor, continually regenerated with dilute sulfuric acid, replaces the sodium ions in the eluent ($NaHCO_3$–Na_2CO_3; see Table 3) with hydrogen ions, thus removing the eluent anions by conversion to carbon dioxide and water. This method permits detection of phosphates in the range 20–100 pmol.

Supercritical Fluid Chromatography

Carbon dioxide, widely regarded as the most useful mobile phase for supercritical fluid chromatography (SFC) is a poor solvent for polar solutes and those having high molecular mass. For this reason such solutes require derivatization to nonpolar products before analysis by SFC is possible. In the carbohydrate field the main successes of the method have been its application to series of homologous oligosaccharides, such as the maltodextrins, as their permethylated or trimethylsilylated derivatives, and to permethylated glycoconjugates. Coupled to chemical ionization mass spectrometry (CI-MS), SFC affords a sensitive analytical method (with detection limits at the picomole level) in such applications as monitoring of degradation of polysaccharides (e.g. starch) and profiling of glycoconjugates. With ammonia as the reactant gas for CI-MS, selected-ion monitoring of the $[M + NH_4]^+$ ions as the analytes emerge from the SFC column permits sensitive detection of derivatized glucooligosaccharides to a degree of polymerization (DP) of 15 and above; for the glycoconjugate derivatives the molecular mass limit is not in

Table 3 LC systems applicable to analysis of carbohydrate derivatives

Compounds	*Column packing*	*Mobile phase*	*Temperature (°C)*	*Detection system*
Acetates	Silica	*n*-Hexane–acetone (10 : 1) or *n*-hexane–ethyl acetate (1 : 1)	RT	RI or UV
Acetylated oligosaccharides (to DP 30–35)	C_{18}-silica	Acetonitrile–water, linear gradient, 10 → 70% acetonitrile (80 min)	65	UV
Alditols	NH_2-silica	Acetonitrile–water (4 : 1)	RT	RI
	Amine-modified silica (impregnated with tetraethylenepentamine)	Acetonitrile–water (3 : 1), containing tetraethylenepentamine (0.02%)	RT	RI
	Cyclodextrin-bonded silica	Acetonitrile–water (4 : 1)	RT	RI
	Cation exchange resin (Ca^{2+} form)	Water	65	RI
	Cation exchange resin (Pb^{2+} form)	Ethanol–water (1 : 4)	80	RI
	Anion exchange resin (quaternary ammonium type)	0.50 mol L^{-1} borate buffer, pH 7.1 (35 min) 0.35 mol L^{-1} borate buffer, pH 8.1 (30 min) 0.50 mol L^{-1} borate buffer, pH 10.5 (25 min)	65	Photometric or fluorimetric automated periodate–petane-2,4-dione method
	Pellicular anion exchanger used in ion chromatography	0.15 mol L^{-1} NaOH	RT	Pulsed amperometric
Aldonic and aldaric acids	Cation exchange resin (H^+ form)	4.5 mmol L^{-1} H_2SO_4	25	UV
	Anion exchange resin (quarternary ammonium type)	0.2 mol L^{-1} ammonium formate, pH 3.2	45	UV
		For aldaric acids: 0.16 mol L^{-1} NaCl containing $MgCl_2$ (20 mmol L^{-1})	85	UV
Amino and acetamidodeoxy-hexoses and -hexitols	NH_2-silica	Acetonitrile–0.15 mmol L^{-1} phosphate buffer, pH 5.2 (4 : 1)	RT	UV
	Cation exchange resin (H^+ form)	Acetonitrile–water (23 : 2)	30	UV, automated 2-cyanoacetamide method
	Cation exchange resin (Na^+ form) used in amino acid analyser	0.1 mol L^{-1} sodium citrate, pH 7.2	40 (15 min); 63 (45 min)	Photometric, automated ninhydrin method
		20 mmol L^{-1} $Na_2B_4O_7$, pH 8.0	60	Fluorimetric, automated *o*-phthalaldehyde method
	Pellicular anion exchanger used in ion chromatography	10 mmol L^{-1} NaOH; post-column addition of 0.3 mol L^{-1} NaOH to raise pH to optimum for detection method	RT	Pulsed amperometric detector
As benzoylated hexitols	Silica	*n*-Hexane–dioxane–dichloromethane, linear gradient, 22 : 2 : 1 → 4 : 2 : 1 (80 min)	RT	UV
As pyridylamino derivatives	C_{18}-silica	0.25 mol L^{-1} sodium citrate buffer, pH 4.0, containing acetonitrile (1.0%)	RT	Fluorimetric

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Table 3 *Continued*

Compounds	*Column packing*	*Mobile phase*	*Temperature (°C)*	*Detection system*
Ascorbic acid	Cation exchange resin (H^+ form)	4.5 mmol L^{-1} H_2SO_4 or 0.1 mol L^{-1} HCOOH	25	UV
			30	Amperometric (glassy carbon electrode)
	C_{18}-silica	25 mmol L^{-1} sodium citrate buffer, pH 4.4, containing *N*-methyldodecylamine (1 mmol L^{-1})	RT	Amperometric, as above
Cyclitols	Amine-modified silica (impregnated with tetraethylenepentamine)	Acetonitrile–water (3 : 1), containing tetraethylenepentamine (0.02%)	RT	RI
	Cation exchange resin (Ca^{2+} form)	Water	85	RI
Gangliosides	NH_2-silica	(A) Acetonitrile–5 mmol L^{-1} phosphate buffer, pH 5.6 (83 : 17) (B) Acetonitrile–20 mmol L^{-1} phosphate buffer, pH 5.6 (1 : 1) Gradient elution: Solution A (7 min); A → A + B (33 : 17) in 53 min; → A + B (9 : 16) in 20 min	RT	UV
Benzoylated	Silica	*n*-Hexane–dioxane, linear gradient, 7 → 23% dioxane (18 min)	RT	UV
Glycolipids, benzoylated	Silica	*n*-Hexane–dioxane, linear gradient, 2.5 → 25% dioxane (13 min); isocratic (5 min)	RT	UV
Glycosides, methyl	Silica	Acetonitrile–water (9 : 1)	RT	RI
	C_{18}-silica	Water	RT	RI
	Cation exchange resin (Ca^{2+} form)	Water	1.5	RI
Benzoylated	C_{18}-silica	Acetonitrile–water, linear gradient, 35 → 90% acetonitrile (65 min)	RT	UV
Gycosides, other				
Acetylated	Silica	Benzene–ethyl acetate (9 : 1) For aryl glycosides: Chloroform–carbon tetrachloride (3 : 2)	RT	UV
	C_{18}-silica	Methanol–water (13 : 7)	RT	UV
Benzoylated	Silica	Benzene–ethyl acetate (99 : 1)	RT	UV
Lactones	Cation exchange resin (H^+ form)	4.5 mmol L^{-1} H_2SO_4	25	UV
Methyl ethers, as alditols	C_{18}-silica	Water–acetonitrile (99 : 1)	RT	RI

Table 3 *Continued*

Compounds	*Column packing*	*Mobile phase*	*Temperature (°C)*	*Detection system*
Neuraminic acid derivatives, KDO and derivatives	Anion exchange resin (quaternary ammonium type)	0.75 mmol L^{-1} Na_2SO_4 For KDO disaccharides: 10 mmol L^{-1} Na_2SO_4	RT	UV
Neuraminic acids, DDB derivatives	C_{18}-silica	Water–methanol–acetonitrile (77 : 15 : 8)	RT	Fluorimetric
Oligogalacturonic acids, to DP10	Cation exchange resin (H^+ form)	5 mmol L^{-1} H_2SO_4	85	RI
DP 5-20	Pellicular anion exchanger used in ion chromatography	0.15 mol L^{-1} NaOH, with sodium acetate, gradient: 0.40 mol L^{-1} (2 min); 0.40 → 0.90 mol L^{-1} (43 min)	RT	Pulsed amperometric
Oligosaccharides, chitin, to DP 5	NH_2-silica	Acetonitrile–water (7 : 3)	25	RI
Oligosaccharides, from glycoproteins	NH_2-silica	Acetonitrile–15 mmol L^{-1} phosphate buffer, pH 5.2 (4 : 1): isocratic (30 min); buffer 20 → 45% (50 min) For higher oligosaccharides : (8–12 sugar residues): Linear gradient: buffer 20 → 44% (80 min)	RT	UV
	Pellicular anion exchanger used in ion chromatography	For neutral oligosaccharides: (2–11 sugar residues): (A) 0.10 mol L^{-1} NaOH (B) 0.10 mol L^{-1} NaOH containing sodium acetate (0.15 mol L^{-1}) Gradient elution: A (10 min) A → A + B (1 : 4) in 60 min Post-column addition of 0.3 mol L^{-1} NaOH to raise pH for detection method For sialylated oligosaccharides (3–8 sugar residues): 50 mmol L^{-1} NaOH containing 100 mmol L^{-1} sodium acetate	RT	Pulsed amperometric
Oligosaccharides (2–8 residues), from hyaluronic acid	NH_2-silica	0.1 mol L^{-1} KH_2PO_4, pH 4.75	RT	UV
Oligosaccharide-alditols, from glycoproteins, 2–7 residues	NH_2-silica	Acetonitrile–1 mmol L^{-1} KH_2PO_4, pH 5.4 (3 : 2)	RT	UV
2–20 residues	Size exclusion chromatography packing	Water	55	RI or scintillation counting after labelling with ^3H

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Table 3 *Continued*

Compounds	*Column packing*	*Mobile phase*	*Temperature (°C)*	*Detection system*
Oligosaccharide-alditols, ethylated and methylated (2–6 residues)	C_{18}-silica	Acetonitrile–water, various proportions from 1 : 1 to 3 : 2, or linear gradient, 50 → 65% acetonitrile (45 min)	RT	RI, MS
Oligosaccharides, pyridylamino derivatives (2–20 residues)	Two-dimensional mapping: (1) C_{18}-silica	For column 1: (A) 10 mmol L^{-1} phosphate buffer, pH 3.8; (B) A containing 1-butanol (0.5%) Linear gradient, 20 → 50%B (60 min)	55	Fluorimetric
	(2) NH_2-silica	For column 2: (C) Acetonitrile–3% acetic acid in water containing triethylamine, pH 7.3 (13 : 7) (D) As C, but proportions 1 : 1 Linear gradient, C → D (50 min)	40	Fluorimetric
Phosphates	Pellicular anion exchanger used in ion chromatography	2.4 mmol L^{-1} $NaHCO_3$–1.92 mmol L^{-1} Na_2CO_3	RT	Conductivity anion micromembrane suppressor
	C_{18}-silica	For monophosphates: 20 mmol L^{-1} HCOOH, containing tetrabutylammonium hydroxide (8 mmol L^{-1}) as ion-pairing reagent and Eu complex (0.02 mmol L^{-1}) as detection reagent For diphosphates: 20 mmol L^{-1} HCOOH–20 mmol L^{-1} HCl–40 mmol L^{-1} NaCl; concentration of tetrabutylammonium hydroxide increased to 30 mmol L^{-1}, that of Eu complex unchanged	38	UV photometry of adduct with Eu complex
Phosphorylated oligosaccharides (2–5 sugar residues)	Pellicular anion exchanger used in ion chromatography	0.1 mol L^{-1} NaOH (5 min); linear acetate gradient, 0 → 0.6 mol L^{-1} sodium acetate in 0.1 mol L^{-1} NaOH (67 min); isocratic (5 min)	RT	Pulsed amperometric
Unsaturated oligosaccharides (2–7 residues), from lyase digestion of:				
Alginate	C_{18}-silica	Acetonitrile–0.1 mol L^{-1} phosphate buffer, pH 6.5 (1 : 9), containing tetrabutylammonium hydroxide (10 mmol L^{-1})	RT	UV

Table 3 *Continued*

Compounds	*Column packing*	*Mobile phase*	*Temperature (°C)*	*Detection system*
Pectic acid	C_{18}-silica	Methanol–0.05 mol L^{-1} phosphate buffer, pH 7.0 (3 : 7), containing tetrabutylammonium bromide (25 mmol L^{-1})	40	UV
	Anion exchanger (quaternary ammonium) bonded to silica	0.4 mol L^{-1} sodium acetate buffer, pH 5.4	40	UV
Hyaluronic acid	C_{18}-silica	(A) Acetonitrile–8 mmol L^{-1} H_3PO_4 (1 : 4), pH of mixture 7.6; contains tetrabutylammonium hydroxide (10 mmol L^{-1})	RT	UV
		(B) Acetonitrile–6 mmol L^{-1} H_3PO_4 (3 : 2), pH of mixture 7.5; concentration of ion-pairing reagent as in A	RT	UV
		Linear gradient, A → A + B (19 : 1) in 18 min		
Unsaturated sulfated oligosaccharides, from lyase digestion of glycosamino-glyronans	Anion exchanger (quarternary ammonium) bonded to silica	For oligosaccharides to hexasaccharide: Linear gradient, 0.2 → 0.8 mol L^{-1} NaCl, pH 3.5 (50 min)	RT	UV
	NH_2-silica	Disaccharides only: 10 mmol L^{-1} Na_2SO_4–1 mmol L^{-1} CH_3COOH	50	UV
	Cation exchange resin (Na^+ form)	Disaccharides only: Acetonitrile–methanol–0.8 mol L^{-1} ammonium formate buffer, pH 4.5 (13 : 3 : 4)	70	UV
Uronic acids	Cation exchange resin (H^+ form)	4.5 mmol L^{-1} H_2SO_4	25	UV
	Anion exchanger (quaternary ammonium) bonded to silica	5 mmol L^{-1} KH_2PO_4 (pH 4.6) containing methanol (5%)	RT	UV
		0.7 mol L^{-1} CH_3COOH	40	RI

RT, Room temperature; RI, refractive index.

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Table 4 Solvent systems useful in TLC and paper chromatography of sugar derivatives

Derivatives	*Stationary phase*	*Solvent system*[a]
Acetals and ketals	Silica gel	Benzene–ethanol (2 : 1) Benzene–acetic acid–ethanol (2 : 2 : 1) For pyruvate: Ethyl acetate–acetic acid–formic acid–water (12 : 3 : 1 : 4) (threefold development)
	HPTLC plates with bonded aminopropyl phase, impregnated with NaH_2PO_4 (0.2 mol L^{-1})	Acetonitrile–water (9 : 1)
Acetates	Silica gel	Benzene–ethyl acetate (7 : 3) Benzene–methanol (9 : 1)
Alditols	Paper	1-Butanol–ethanol–water (4 : 1 : 5), upper layer 2-Butanone–acetic acid–saturated aqueous solution of H_3BO_3 (9 : 1 : 1)
	Paper impregnated with tungstate (0.15 mol L^{-1})	Acetone–1-butanol–water (5 : 3 : 2)
	CMC paper (La^{3+}, Ca^{2+} or Ba^{2+} forms)	1-Butanol–ethanol–water (10 : 1 : 2)
	Cellulose plates impregnated with tungstate (0.15 mol L^{-1})	Acetone–1-butanol–water (5 : 3 : 2)
	Silica gel impregnated with NaH_2PO_4 (0.5 mol L^{-1})	2-Propanol–acetone–0.2 mol L^{-1} lactic acid (6 : 3 : 1)
Aldonic acids and aldonolactones	Cellulose plates	1-Butanol–acetic acid–water (6 : 1 : 2)
	Silica gel, HPTLC	Ethyl acetate–pyridine–acetic acid–tetrahydrofuran–water (50 : 22 : 4 : 15 : 15)
Amino- and acetamidodeoxyhexoses	Paper	Ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3) 1-Butanol–pyridine–benzene–water (5 : 3 : 1 : 3)
	Cellulose plates	1-Butanol–pyridine–0.1 mol L^{-1} HCl (5 : 3 : 2) Two-dimensional development: (1) 2-Propanol–90% HCOOH–water (20 : 1 : 5); (2) Lutidine–water (13 : 7)
	Silica gel	1-Propanol–water (7 : 1)
Dansyl derivatives	Silica gel	Cyclohexane–ethyl acetate–ethanol (6 : 4 : 3)
Anhydroalditols	Silica gel, HPTLC, impregnated with borate (0.1 mol L^{-1})	1-Butanol–acetone–water (5 : 4 : 1)
Anhydro sugars	HPTLC plates with bonded aminopropyl phase, impregnated with NaH_2PO_4 (0.2 mol L^{-1})	Acetonitrile–water (9 : 1)
Branched-chain sugars (apiose, hamamelose and derivatives)	Paper	1-Butanol–pyridine–acetic acid–water (60 : 40 : 3 : 30) 1-Butanol–ethyl acetate–acetic acid–water (8 : 6 : 5 : 8)
Cyclitols	Paper	Acetone–water (4 : 1)
Gangliosides	Silica gel, HPTLC	Methyl acetate–2-propanol–33 mmol L^{-1} KCl (9 : 6 : 4) Acetonitrile–2-propanol–50 mmol L^{-1} KCl (10 : 67 : 23) Acetonitrile–2-propanol–2.5 mol L^{-1} aqueous ammonia (2 : 13 : 5)
Glycosides, aryl, acetylated	Silica gel	2-Butanone–light petroleum (1 : 3)

Table 4 *Continued*

Derivatives	*Stationary phase*	*Solvent system*[a]
Glycosides, methyl	Paper	*t*-Pentanol–1-propanol–water (8 : 2 : 3)
	Cellulose plates	Ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3)
		1-Butanol–acetic acid–water (3 : 1 : 1)
Methylated	Silica gel	Benzene–ethanol–water (170 : 47 : 15), upper layer
Methyl ethers	Paper	1-Butanol–ethanol–water (4 : 1 : 5), upper layer
		2-Butanone–water azeotrope (85 : 7)
	Cellulose plates	2-Butanone–saturated with water
	Silica gel	2-Butanone–water azeotrope
		Benzene–ethanol–water–aqueous ammonia (200 : 47 : 15 : 1), upper layer
	Silica gel impregnated with H_3BO_3 (0.1 mol L^{-1})	1-Butanol–acetone–water (4 : 5 : 1)
Muramic acid (separated from aminodeoxyhexoses)	Cellulose plates	Two-dimensional development : (1) 2-Propanol–90% HCOOH–water (20 : 1 : 5), (2) Lutidine–water (13 : 7)
	Silica gel	Acetonitrile–ethanol–acetic acid–water (13 : 2 : 1 : 4)
Neuraminic acids, *N*-acetyl and *N*-glycolyl	Silica gel	Methanol–water (5 : 2)
Oligogalacturonic acids (to DP 9)	Cellulose plates	Ethyl acetate–acetic acid–water (2 : 1 : 2), twofold development
	Silica gel, HPTLC	Ethanol–25 mmol L^{-1} CH_3COOH (21 : 29), 35°C
Oligosaccharides, chitin (to DP 6)	HPTLC plates with bonded aminopropyl phase, impregnated with NaH_2PO_4 (0.2 mol L^{-1})	Acetonitrile–water (18 : 7)
Oligosaccharides, from hyaluronic acid (2–8 residues)	Silica gel	2-Propanol–water (33 : 17), containing NaCl (50 mmol L^{-1})
Phosphates	Paper	Methanol–90% HCOOH–water (16 : 3 : 1), containing tetrasodium salt of EDTA (0.05%, m/v)
Unsaturated disaccharides, from lyase digestion of glycosaminoglycuronans, run as dansylhydrazones	Silica gel	1-Propanol–2-propanol–1-butanol–water (6 : 9 : 1 : 4), containing NaCl (40 mmol L^{-1}) and ammonia (10 mmol L^{-1})
Uronic acids and alduronolactones	Paper	1-Butanol–acetic acid–water (2 : 1 : 1)
		Ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4)
		Ethyl acetate–acetic acid–pyridine–water (10 : 3 : 3 : 2)
	DEAE-cellulose paper	Ethyl acetate–acetic acid–water (3 : 1 : 1)
	Cellulose plates	1-Butanol–acetic acid–water (6 : 1 : 2)
	Silica gel impregnated with NaH_2PO_4 (0.3 mol L^{-1})	1-Butanol–ethanol–0.1 mol L^{-1} H_3PO_4 (1 : 10 : 5)

[a]All proportions are by volume.

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Table 5 Lectins used in affinity chromatography of oligosaccharides

Lectin	*Specificity*
Concanavalin A (Con A)	α-D-Man, terminal or substituted only at *O*2; terminal β-D-GlcNAc at *O*2 promotes binding
Datura stramonium agglutinin (DSA)	[β-D-Gal (1 → 4) β-D-GlcNAc (1 → 3)]$_n$, i.e. poly (*N*-acetyllactosamine); binds tri- and tetraantennary oligosaccharides lacking this sequence if outer Man residue is substituted at *O*2 and *O*6 by *N*-acetyllactosamine
Griffonia simplicifolia	Terminal α-D-Gal
Helix pomatia (*HP*)	Terminal α-D-GalNAc
Lens culinaris (lentil)	Terminal α-D-Man: outer Man residue substituted at *O*2 and *O*6 by GlcNAc
Phytohaemagglutinin, erythroagglutinating (E_4-PHA)	Bisecting GlcNAc at *O*4 of inner Man residue and sequence β-D-Gal (1 → 4) β-D-GlcNAc (1 → 2) α-D-Man in outer chains
Phytohaemagglutinin, leukoagglutinating (L_4-PHA)	Tri- and tetraantennary oligosaccharides with outer Man residue substituted at *O*2 and *O*6 by *N*-acetylactosamine
Pisum sativum (pea)	Terminal α-D-Man; α-L-Fuc at *O*6 of 4-linked GlcNAc in inner core of *N*-linked oligosaccharide
Ricinus communis agglutinin (RCA-I)	Terminal β-D-Gal
Sambucus nigra	α-NeuAc (2 → 6) Gal ≫ α-NeuAc (2 → 3) Gal
Wisteria floribunda	β-D-GalNAc (1 → 4) Gal > α-D-GalNAc (1 → 3) Gal and α-D-GalNAc (1 → 3) GalNAc: substitution of 4-linked Gal by NeuAc at *O*3, or of 3-linked Gal by α-L-Fuc at *O*2 weakens binding

excess of 5000. For these the addition of methanol to the carbon dioxide mobile phase has proved advantageous. Fused-silica microbore capillary columns, with a bonded methylpolysiloxane stationary phase (DB-1 and, especially, DB-5 are very effective), are used at temperatures ranging from 90 to 120°C and with pressure programming over the range 10–40 MPa (100–400 bar) at about 0.5 MPa min^{-1} (5 bar min^{-1}). Under these conditions there is resolution of α and β anomers (more pronounced with the TMS derivatives) and fine structure is discernible in glycoconjugates.

Thin-Layer Chromatography (TLC) and Paper Chromatography

While nonpolar derivatives can be separated by thin-layer chromatography (TLC) on unmodified silica plates, resolution of polar molecules is generally poor unless the silica gel layer is impregnated beforehand with an inorganic salt capable of interacting with carbohydrates. Borate or phosphate buffers are most often used for this purpose; tungstate can also prove effective, especially in TLC of alditols. The same applies to TLC on high performance (HP) TLC plates, particularly those carrying a bonded aminopropyl phase, which is liable to react covalently with sugars and derivatives containing hydroxyl groups. Some separations, particularly those of aminodeoxy sugars, that are not well resolved on impregnated silica gel plates, are better on unmodified silica plates. Cellulose plates also give satisfactory resolution of these derivatives, and of neutral sugars and uronic acids, but two-dimensional development is often required. Impregnation of these plates with tungstate greatly improves their resolving power for alditols.

Although paper chromatography has largely been superseded by TLC, there are groups of sugar derivatives that are far better resolved on paper than by TLC methods. The mixtures of partially methylated sugars obtained in methylation analysis of polysaccharides afford a prime example: resolution on cellulose plates is better than that on silica plates but

paper chromatography remains the most effective method. As in the case of TLC, separation of alditols on paper is improved by impregnation of the paper with tungstate. Papers having ion exchange properties can also be used to good effect in separations of some sugar derivatives: uronic acids and aldobiouronic acids are well resolved on DEAE–cellulose paper (anion exchanger), while carboxymethylcellulose paper, converted beforehand to the La^{3+}, Ca^{2+} or Ba^{2+} forms, gives excellent resolution of alditols.

Some solvent systems that have proved effective in TLC and paper chromatography of sugar derivatives are listed in **Table 4**.

Affinity and Enzyme Methods

Affinity Chromatography

Lectin affinity chromatography is a valuable technique in analyses of glycoconjugates, as the isolation and identification of glycopeptides and the various oligosaccharides obtained on removal of the carbohydrate side chains from the protein or lipid moieties are greatly facilitated by chromatography on a series of short columns, each containing a different lectin covalently coupled to agarose gel. The lectins are selected according to their specificity towards carbohydrates having certain of the main structural features found in the oligosaccharides, and in this way the complex mixture of oligosaccharides can be fractionated according to structure. Some of the lectins that have proved useful in such studies are listed in **Table 5**, together with their carbohydrate-binding specificities.

The oligosaccharides are usually applied to the lectin columns in phosphate-buffered saline (PBS), pH 7.2, Tris-buffered saline (TBS), pH 8.0, or 10 mmol L^{-1} Tris–HCl buffer, pH 7.5; sodium azide (0.02%, m/v) is added as a preservative and small amounts of calcium, magnesium and manganese chlorides (1 mmol L^{-1}) are essential to the binding action of some lectins, notably concanavalin A. Oligosaccharides that are not bound or are only retarded on the lectin column are eluted with these buffers, but those that are strongly bound require the addition of a competing hapten to the eluent. Haptens applicable to the lectins listed above include methyl α-D-mannopyranoside, lactose, GalNAc, GlcNAc and *N*,*N*′-diacetylchitobiose.

A recent development in affinity chromatography is the use of monoclonal antibodies as ligands; these are highly specific but less strongly reactive than lectins, and the dissociation constants of the complexes formed with bound solutes are sufficiently low to permit rapid fractionation, the oligosaccharides reacting with the ligand being merely retarded on the column, not totally immobilized. An example of the use of this technique is afforded by the complete separation of two of the oligosaccharides of human milk, α-NeuAc(2 → 3)β-D-Gal(1 → 3)β-D-GlcNAc(1 → 3)β-D-Gal(1 → 4)Glc (lactosialyltetrasaccharide, LSTa) and that designated sialyl Le^a, which carries α-L-Fuc at O4 of GlcNAc. On a short column containing monoclonal antibody 19.9 coupled to agarose gel, with 10 mmol L^{-1} Tris–HCl buffer, pH 7.5, as eluent, the two oligosaccharides are rapidly separated, the fucosylated sialyl Le^a being the more retarded. The active oligosaccharides of blood group A are similarly fractionated according to chain lengths and degree of fucosylation by chromatography on immobilized IgM antibody, with TBS as eluent. Use of columns in which such antibodies are coupled to microparticulate silica makes possible very rapid separations of oligosaccharides (in 20 min or less). This new technique of high performance liquid affinity chromatography (HPLAC) has great potential in applications such as clinical analysis, for which methods that are highly specific but also efficient are required.

Enzyme Methods

Enzyme methods are particularly useful in analyses of glycoconjugates, for the release of mono- or oligosaccharides that are not easily liberated by acid hydrolysis or are acid-labile, and in the determination of some constituents. The determination of neuraminic acid derivatives in glycoproteins or glycolipids is a striking example of this use of enzymes. The sample (~200 μg), dissolved in 60 mmol L^{-1} phosphate buffer (pH 7.0, 800 μL), is incubated at 37°C for 1 h with *Clostridium perfringens* neuraminidase (EC 3.2.1.18) and *N*-acylneuraminate pyruvate-lyase (EC 4.1.3.3). The former (0.5 U) liberates the neuraminic acid derivatives from glycosidic linkages and the latter (0.3 U) cleaves the molecules to produce *N*-acylmannosamines and pyruvate. The mannosamine derivatives are well separated from GlcNAc, GalNAc and neutral sugar components of glycoconjugates by LC (H^+ form cation exchange resin, 92% acetonitrile in water; see Table 3) and may be determined in this way. Alternatively (or in addition), the proportion of neuraminic acids may be found by determining the pyruvate released, using the definitive lactate dehydrogenase method.

For release of *N*-linked oligosaccharides from glycoproteins, digestion with *N*-oligosaccharide glycopeptidase (EC 3.5.1.52) offers a milder alternative to the standard hydrazinolysis procedure. After pepsin digestion of the protein moiety, the product, dissolved in 0.1 mmol L^{-1} citrate–phosphate buffer,

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is digested with the glycopeptidase (1 mU per 1000 nmol of oligosaccharides) at 37°C for 15 h. For sequencing purposes, smaller oligosaccharides may be obtained by subsequent digestion with various exoglycosidases, such as α-L-fucosidase (EC 3.2.1.51), β-D-galactosidase (EC 3.2.1.23) and β-*N*-acetylglucosaminidase (EC 3.2.1.30). The mixtures of oligosaccharides are separated by LC (see Table 3).

Enzyme methods are also important in the analysis of glycosaminoglycuronans, which are very resistant to acid hydrolysis. Hyaluronidase (EC 3.2.1.35) randomly cleaves the (1 → 4) bonds linking the acetamidodeoxyhexose residues to glucuronic acid in both hyaluronic acid and the chondroitin sulfates, to yield the disaccharide repeating unit and oligomers. An exception to this is leech hyaluronidase (EC 3.2.1.36), which specifically cleaves the β-D-GlcA (1 → 3)β-D-GlcNAc linkages in hyaluronic acid, yielding a different series of oligomers. All of these, including some with odd numbers of sugar residues, obtained by removal of the nonreducing GlcA end-groups with β-glucuronidase (EC 3.2.1.31) or of nonreducing GlcNAc end-groups with β-*N*-acetylglucosaminidase, are well separated by LC (see Table 3).

A sensitive analytical method for glycosaminoglycuronans is afforded by LC of the unsaturated oligosaccharides produced on digestion with enzymes having lyase activity, which give disaccharides or, in the case of hyaluronic acid, tetra- and hexasaccharides with 4,5-unsaturated residues (4-deoxy-L-*threo*-hex-4-enopyranosyluronic acid from D-glucuronic acid or L-iduronic acid) at their nonreducing ends. Chondroitinase ABC (EC 4.2.2.4) digests chondroitin 4- and 6-sulfate and dermatan sulfate, whereas chondroitinase AC (EC 4.2.2.5) does not act upon dermatan sulfate, and LC analysis (Table 3) of the mixtures of unsaturated, sulfated disaccharides produced by each enzyme permits quantification of the respective parent glycosaminoglycuronans. Typically, the proteoglycan (1–1000 μg) is digested at 37°C for 16 h with the enzyme (0.05 U) in Tris buffer (pH 6.0). Hyaluronic acid can be determined specifically by LC analysis of the unsaturated tetra- and hexasaccharide produced on digestion with the lyase from *Streptomyces hyalurolyticus* (H-1136), which cleaves this polymer selectively. Recently the LC profiles of the products of digestion of heparin with heparin lyase (EC 4.2.2.7), from *Flavobacterium heparinum*, have been suggested as a means of characterizing this polydisperse glycosaminoglycuronan: di-, tetra- and hexasaccharides, differing in degree of sulfation and proportion of iduronic acid, are produced, their proportions in the mixture varying with the source of the heparin.

Structural analysis of alginates, which contain blocks of mannuronic acid and guluronic acid residues, all (1 → 4) linked, is facilitated by the use of enzymes acting exclusively on one of these acids, leaving intact blocks of the other. These enzymes are lyases, producing unsaturated oligosaccharides from the portions of the polymer that they attack. For example, a β-D-mannuronase has been isolated from actively growing tissues of the seaweed *Sargassum fluitans* and an α-L-guluronase from the bacterium *Klebsiella aerogenes* type 27. Digestion may be monitored by LC analysis of the unsaturated oligosaccharides (Table 3). This applies also to digestion of pectic acid with *endo*-polygalacturonic acid lyase (EC 4.2.2.10). The saturated oligogalacturonic acids produced on digestion of this polymer with *endo*-polygalacturonase (pectinase: EC 3.2.1.15) are also analysed by LC.

Specific Problems: Analysis of Acidic Derivatives

Whereas the enzyme methods discussed above are used in the degradation of glycoproteins and glycolipids, which contain sugar derivatives – such as the neuraminic acid derivatives – that are unstable when heated in acid, and of glycosaminoglycuronans and polyuronans, which are strongly resistant to acid hydrolysis, it is the latter technique that is most widely used to liberate the constituent monosaccharides from other heteropolysaccharides. For those containing aldobiouronic acid linkages, which are far less readily hydrolysed than are glycosidic linkages between neutral sugars, slow release and low yields of both hexuronic acids and the contiguous (interior) sugar residues make quantification difficult. The use of vigorous conditions or prolonged exposure to acid in attempting to improve the yields of these constituents is liable to cause both decarboxylation of the acid and decomposition of some of the neutral sugars already liberated (pentoses being especially vulnerable). For quantitative GC analysis, the difficulty can be obviated by prior reduction of the carboxyl groups in the uronic acid compounds. This is best effected by treatment with a carbodiimide at pH 4.75, followed by reduction with sodium borohydride or borodeuteride at pH 7.0; if the latter is used, the hexoses produced from the hexuronic acids are labelled with deuterium and thus readily identifiable by GC-MS of the derived alditol acetates.

In methylation analysis, the problems posed by resistance to acid hydrolysis of linkages involving methylated uronic acid residues (present as methyl esters) are similar. In this case the recommended procedure is reduction of the carboxylate ester groups

with lithium aluminium deuteride in dry oxolane (tetrahydrofuran) at 70°C for 16 h. The ester residue is then converted to a 6,6-dideuteriohexose residue, the O-methyl ethers of which are easily distinguishable by GC-MS of the derived alditol acetates.

An alternative to acid hydrolysis that is applicable to most polysaccharides and glycoconjugates, including those containing acid-labile residues or glycosidic linkages resistant to hydrolysis, is afforded by methanolysis, in which the sample is heated in methanolic HCl, the conditions employed depending upon the nature of the sugar residues present. After suitable derivatization, all components of methanolysates, now present as methyl glycosides or, in the case of hexuronic acids, methyl glycoside methyl esters, can be analysed simultaneously, either by GC (Table 2) or by LC (Table 3). The procedure is also applicable to methylation analysis, the methylated methyl glycosides and methyl glycoside methyl esters being amenable to GC without further derivatization (Table 2).

See Colour Plate 118.

See also: **II/Chromatography:** Paper Chromatography. **Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry. **Chromatography: Liquid:** Derivatization. **Chromatography: Thin-Layer (Planar):** Spray Reagents. **III/Impregnation Techniques: Thin-Layer (Planar) Chromatography. Polysaccharides:** Centrifugation; Liquid Chromatography.

Further Reading

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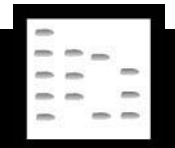
SULFUR COMPOUNDS: GAS CHROMATOGRAPHY



W. Wardencki, Technical University of Gdańsk, Gdańsk, Poland

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Introduction

Sulfur compounds, of both biogenic and anthropogenic origin, constitute a large group of compounds, ranging from simple gases up to complex polycyclic aromatics. These compounds can be present in various, usually complex matrices, such as air (gaseous), water systems (aqueous), various petroleum fractions (gaseous, liquid and solid), in beverages and foodstuffs and in pharmaceutical formulations.

Environmentalists believe that these compounds are responsible for the damage of our environment through acid deposition, rapid acidification of lakes, the loss of forests, the corrosion of metal structures and historical monuments. The interest in biogeochemistry results from the role some sulfur compounds play in global chemical cycles. Dimethyl sulfide (DMS) in sea water, produced in the oceans, is believed to play a critical role in the global sulfur cycle and the radiation balance of the Earth. Also, other sulfur compounds may contribute significantly to the sulfur flux in the atmosphere. In foods, beverages and in water, trace levels of sulfur-containing compounds are responsible for taste and odour problems. They are also the source of malodorous conditions in municipal sewage systems. Refiners worldwide give particular attention to these compounds because in petrochemical and chemical applications even trace levels of sulfur impurities may cause concern. They can poison the catalysts, impart